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Studies have shown that apoptosis and survival pathways in response to DNA damage play a critical role in breast cancer development and progression. 90% of breast cancer cases are sporadic where mutations of BRCA1/2 have not been detected. Other breast cancer genes must exist. Our group has approached the issue in two ways, a genomic and a proteomic approach. We have established and utilized a novel retrovirus-based genetic screen system to search for genes that would confer resistance to DNA dmage induced apoptosis. Multiple clones have been isolated from this genetic screen. Among the genes identified are both novel and known proteins that may be important in DNA-damage responses. To further elucidate the pathways mediated by BARD1, we looked for factors interacting with BARD1 using mass spec sequencing. Several factors have emerged from this study and are being examined. The information obtained from our studies should prove useful for developing new and effective screening strategies, drug targets, and treatment for breast cancer.

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Introduction

Understanding the molecular and cellular mechanisms that trigger breast cancer is essential to the prevention and treatment of this disease. The initiation and progression of breast cancer is likely the result of dysregulation of both oncogenes and tumor suppressor genes (1, 2). Mutations of these genes can cause defects in cellular survival and proliferation, genomic integrity, and sensitivity to DNA damage. However, few genes that regulate DNA damage induced cell death are known to date, and even less is known as to how they interconnect with the apoptosis and survival pathways. We have proposed to establish a genetic system to screen for genes that regulate survival in cultured cells through high-efficiency mutagenesis using Enhanced Retroviral Mutagens (ERM) (3). Due to the random nature of retroviral integration, endogenous genes involved in cell survival signaling cascades may be activated or inactivated by ERM. The targeted gene loci are marked by retroviral integration thereby allowing quick isolation of the candidate genes. The overall objective of this proposal is to identify and study genes that allow the survival of normal and cancerous breast cells. The physiological roles of these genes and their interactions with known signaling pathways will be investigated. Genetic screens will be performed to search for survival genes in response to DNA damage. And the function and signaling mechanisms of BARD1 in breast cancer cell survival will also be examined. The proposed studies should help in our understanding of the molecular basis underlying cell survival signaling and breast cancer as well as provide new therapeutic targets for the cure of this disease.

Body

For Task 1, we have proposed to isolate mammalian genes involved in breast cancer cell survival. This will be achieved by establishing Enhanced Retrovirus Mutagen (ERM)-mediated genetic screen and analyzing isolated clones, by establishing secondary screens using human breast epithelial cells to confirm the role the cloned genes in DNA-damage-induced apoptosis, and by identifying the candidate genes targeted by ERM

In the last report, we described the employment of a novel retrovirus-based genetic screen system (3) to ientify genes that mediate DNA-damage induced apoptosis. Briefly, cytokine-dependent cells were infected with the mutagenesis virus, and selected in the presence of the DNA-damage inducing drug cisplatin (CDDP). Multiple clones have been isolated from this genetic screen. In Table 1, we listed a number of genes that we have identified to date. We are carrying out further studies to clarify the genes that are most relevant to our study.

Genes	Possible function
novel	Conserved through evolution. Binds to RAD50
NESH	Homologue of Abi-1. Sh3 domain containing protein
novel	Unknown function
Lyn	Tyrosine kinase. Involved in DNA-damage response (4-7).

Table 1.

For Task 2, we have proposed to biochemically characterize breast cancer genes including how BARD1 may be involved in breast cancer cell survival. BARD1 was cloned originally as an interactor of BRCA1 and has been implicated as a critical factor in BRCA1 tumor suppression (8). Missense, point mutation and loss-of-function mutations of BARD1 have been found in breast cancers (9). Our preliminary results suggest that BARD1 may modulate cell survival in response to DNA damage. One aspect of the study was to identify the factors that may interact with BARD1. To accomplish this, we took a proteomic approach (Figure 1).

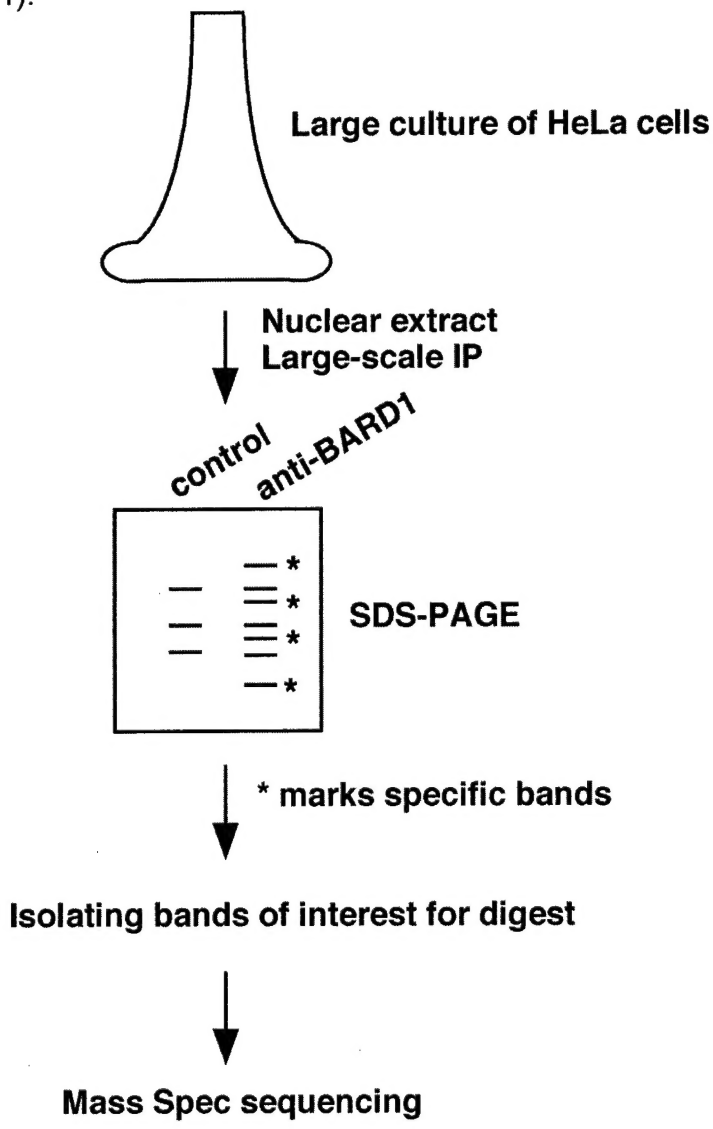

Figure 1. Flow chart for identification of proteins interacting with BARD1

Briefly, large amount of human HeLa cells were grown and harvested. Nuclear extracts were made from these cells and then immunoprecipitated in large-scale with an anti-BARD1 antibody. The immunoprecipitates were subsequently resolved by SDS-

PAGE, and visualized by Coomassie Blue Staining. Desired bands were then excised from the gels, digested, and sequenced via Mass Spectrometry.

Through this method, we have identified a number of factors known to interact with BARD1 complexes (Table 2). Furthermore, three novel factors were also identified. And we are actively pursueing these studies.

Genes	Known Function
CPSF-1	multisubunit cleavage and polyadenylation specificity factor (10,1
CPSF-7	multisubunit cleavage and polyadenylation specificity factor (10,1
Symplek	Complexes with BARD1 associated proteins (12).

Table 2.

Key Research Accomplishments

- Generation of CDDP resistant 32D cell clones
- Establishment of a system to identify BARD1 associated factors
- Successful identification of a number of novel factors from the genetic screen
- Successful identification of a number of novel factors from the proteomic approach

Reportable Outcomes

We have successfully utilized the ERM approach to identify genes that would confer resistance to CDDP induced cell death. Among the genes identified are both novel and known factors. We have also used a proteomic approach to identify factors in the BARD1 complex. We are currently working on a number of novel proteins in this complex.

Conclusions

In summary, we have improved and successfully utilized our genetic screen approach for high efficiency mutagenesis. Multiple clones have been isolated and some of the gene loci targeted by the ERM mutagen have also been identified. Furthermore, we are on our way to elucidate how BARD1 may affect cell survival through its interaction with other factors. The information obtained from our studies should prove especially useful for the development of new and effective screening strategies, drug targets, and treatment for breast cancer.

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